Meeting Viewpoint

Workshop Report for Cancer Research: Defining the Shades of Gy: Utilizing the Biological Consequences of Radiotherapy in the Development of New Treatment Approaches

Mansoor M. Ahmed[1], C. Norman Coleman[1,2], Marc Mendonca[3], Soren Bentzen[4], Bhadrasain Vikram[1], Stephen M. Seltzer[5], Dudley Goodhead[6], Ceferino Obcemea[1], Radhe Mohan[7], Kevin M. Prise[8], Jacek Capala[1], Deborah Citrin[2], Gary Kao[9], Molykutty Aryankalayil[2], Iris Eke[2], Jeffrey C. Buchsbaum[1], Pataje G.S. Prasanna[1], Fei-Fei Liu[10], Quynh-Thu Le[11], Beverly Teicher[12], David G. Kirsch[13], DeeDee Smart[2], Joel Tepper[14], Silvia Formenti[15], Daphne Haas-Kogan[16], David Raben[17], and James Mitchell[18]

INTRODUCTION

The ability to physically target radiotherapy using image-guidance is continually improving with photons and particle therapy that include protons and heavier ions such as carbon. The unit of dose deposited is the gray (Gy); however, particle therapies produce different patterns of ionizations, and there is evidence that the biological effects of radiation depend on dose size, schedule, and type of radiation. This National Cancer Institute (NCI)–sponsored workshop addressed the potential of using radiation-induced biological perturbations in addition to physical dose, Gy, as a transformational approach to quantifying radiation.

[1]Radiation Research Program, National Cancer Institute, Bethesda, Maryland. [2]Radiation Oncology Branch, Centre for Cancer Research, National Cancer Institute, Bethesda, Maryland. [3]Department of Radiation Oncology, Indiana University School of Medicine, Indianapolis, Indiana. [4]Department of Epidemiology, University of Maryland School of Medicine, Baltimore, Maryland. [5]Radiation Physics Division, NIST, Gaithersburg, Maryland. [6]Medical Research Council, Harwell, Didcot, United Kingdom. [7]Division of Radiation Oncology, Department of Radiation Physics, The University of Texas, MD Anderson Cancer Centre, Houston, Texas. [8]Centre for Cancer Research and Cell Biology, Queen's University Belfast, Belfast, United Kingdom. [9]Department of Radiation Oncology, University of Pennsylvania, Philadelphia, Pennsylvania. [10]Department of Radiation Oncology, University of Toronto, Princess Margaret Cancer Centre, Toronto, Canada. [11]Department of Radiation Oncology, Stanford University School of Medicine, Stanford, California. [12]Development Therapeutics Program, National Cancer Institute, Bethesda, Maryland. [13]Department of Radiation Oncology, Duke University School of Medicine, Durham, North Carolina. [14]Department of Radiation Oncology, University of North Carolina School of Medicine, Chapel Hill, North Carolina. [15]Department of Radiation Oncology, Weill Cornell Medical College, New York City, New York. [16]Department of Radiation Oncology, Harvard Medical School, Dana-Farber Cancer Institute, Boston, Massachusetts. [17]Department of Radiation Oncology; University of Colorado Cancer Center, Aurora, Colorado. [18]Radiation Biology Branch, National Cancer Institute, Bethesda, Maryland.

M.M. Ahmed and C.N. Coleman had equal contribution in the conceptualization of the workshop and preparation of the report.

Corresponding Authors: C. Norman Coleman, National Cancer Institute, NIH, 9609 Medical Center Drive, Bethesda, MD 20892-9727. Phone: 240-276-5690; Fax: 240-276-5827; E-mail: ccoleman@mail.nih.gov; and Mansoor M. Ahmed, ahmedmm@mail.nih.gov

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New directions included:

- Radiation effects on the cell, tumor, and normal tissue can be exploited so that schedules beyond the conventional and still useful 2 Gy fraction can be utilized. Different radiation dose, type, and schedule (multifraction) can potentially act "as a drug," with unique and exploitable mechanism of action. This concept pertains to radiation alone and with molecular-targeted therapy and immunotherapy.
- Utilizing biomarkers of radiotherapy in precision medicine to assess both treatment efficacy and normal tissue damage, potentially including organ-specific biomarkers of tissue damage.

Controversies to:

- Defining the most resistant subpopulation within a tumor, particularly at higher fractional doses. Ameliorating resistance could include targeting tumor cells and/or the vasculature.
- Rethinking the target and extent of tumor volume irradiated as potential new strategies for enhancing curative benefit and avoiding normal tissue toxicity with preservation of organ function.

Current challenges in:

- Defining radiation parameters for selected target induction, such as a survival pathway, tumor antigen, enhanced immune response, or change in vasculature.
- Determining the duration in the change of radiation-inducible phenotype to be exploited.
- Eventually modifying the treatment from the "time-honored" approaches.

Altogether, these approaches can facilitate the incorporation of radiation technology and biology into treatment strategies for personalized medicine, including molecular and immune-targeted therapies.

REPORT

The National Cancer Institute's Radiation Research Program hosted the workshop "Shades of Gy (gray): Biological Consequences of Radiation Therapy" on September 11–12, 2017 (agenda; https://dctd.cancer.gov/NewsEvents/

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biological_consequences_of_radiation_therapy_workshop_agenda.pdf). The focus is a novel concept of defining radiation dose both in energy deposition (Gy) and biologically meaningful perturbations in the tumor and normal tissue. This is intended to broaden the application of radiotherapy in precision medicine. The workshop's long-term goal is to build from a combination of well-known reliable models, new cancer biology, and clinical experience to develop new paradigms for clinical cancer care.

Norman Coleman (NCI) opened the workshop stressing the potential use of radiation as a drug with radiation dose described in appropriate units similar to drug therapy such as pharmacokinetics and pharmacodynamics. This requires returning to fundamental processes including the mechanism(s) of radiation damage, biophysics, stress and adaptive responses, and tumor microenvironment effects under the umbrella of "Accurate, Precision Radiation Medicine." The importance of physical dose remains paramount. Emphasizing ambiguous terminology in use is that the unit "gray" used in biodosimetry is actually a biological change (a "biodose") and not the physical dose. The new paradigm for biological dose in addition to physical dose uses modern molecular endpoints that can improve cancer care.

Several challenging issues on current radiation "Dose-Effect Models" were discussed in Session I. Soren Bentzen (University of Maryland, Baltimore, MD) stressed the importance of a normalized dose-response gradient that would allow the tumor control probability/normal tissue complication probability percentage versus total radiation dose when given as 1.8 Gy per fraction to be predicted for radiation plus chemotherapy or immunotherapy and all three treatments combined. He suggested the use of the linear quadratic (LQ) $EQD_2\alpha/\beta$ to predict actual clinical outcome in patients with head and neck squamous cell cancer and to compare the LQ model with treatment biomarkers ranging from biological to imaging markers that can provide integral effects of dose and time for tumor and normal tissue. This approach could provide lead time for changes in treatment, thereby improving both cancer treatment outcomes and quality of life. Dr. Martin Brown (Stanford University, Stanford, CA) supported the use of the LQ model and pointed out key exceptions to their use, such as the role of the tumor stroma and tumor vasculature in determining tumor radiation sensitivity, for which the endpoint TCD50 (dose to control 50% of the murine tumors irradiated) should be adopted. He asserted that radiosensitivity of tumors is only relevant to tumor control if the radiation-damaged vasculature is restored by bone marrow–derived cells (1). Blocking the SDF-1/CXCR4 pathway would block the bone marrow–derived cell's recruitment and increase tumor control, with the added benefit of protecting normal tissues. Philip Lambin (Maastricht University, Maastricht, the Netherlands) discussed that the LQ models should be revisited to understand whether low or high α/β-ratio tumors can be explained with modern molecular biological endpoints, and he also suggested to study whether a tumor is slowly or rapidly proliferating. He proposed that to help predict treatment response and overall survival, quantitative imaging radiomics (with three-dimensional dose delivery and four-dimensional radiomics) would enable the measure of proliferation signatures and other tumor parameters that would help link classical α/β concepts with relevant biology such as DNA repair, proliferation, and reoxygenation.

That basic biophysics provides a great deal of information on radiation effects and also supports the need for biological assessment to enhance physical dose was discussed in Session II. The track structure patterns of ionization and excitation from various radiation particle types and their secondary charged particles in complex biological media (cells in suspension or in tissue and specific subcellular targets) are not clear, particularly at low incident energies, where the energy transfer is most pronounced. Dudley Goodhead (Medical Research Council, London, United Kingdom) and Jan Schuemann (Massachusetts General Hospital, Boston, MA) discussed the latest progress in Monte Carlo simulations of track–structure interactions with biological structures and concluded that at the nanometer level of cellular targets, radiation dose, dose rates, and fractions in radiotherapy would have to be so much higher than those used clinically to have overlaps of multiple primary tracks in the target volumes involved in the initial physical damage. Overall, track structure provides physical data that indeed inform microdosimetry, but cellular response requires much more knowledge of biological response functions, such as relative biological effectiveness, RBE (the RBE is defined as the ratio of the doses required by two types of radiation to cause the same level of effect). Thus, the RBE depends on the dose and the biological endpoint. Lisa Cornell (NASA, Norfolk, VA) and Francis Cuccinota (University of Nevada, Las Vegas, Las Vegas, NV) reviewed the importance of track structure coupled with biophysical models in space radiation protection in which heavy ions present the major biological threats to astronauts. In summary, track structure provides the qualitative understanding of the different RBEs from the variety of radiation modalities used in therapy, and with the addition of biological-response models, track-structure results can be more realistically extrapolated to estimate the biological effects of any incident radiation.

Session III transitioned from physical dose to biological effects, focusing on the RBE of particles compared with photons. The rationale for defining RBE in this manner as a ratio is to allow extrapolation of the vast clinical experience with photon therapy to particle treatment. It is argued that the very concept of RBE is questionable because, biologically, protons and heavier ions are very different from photons. Kevin Prise (Queen's University Belfast, Belfast, Northern Ireland, United Kingdom) stressed the fact that one of the sources of uncertainty of RBE is the biological effects of the reference photon. For protons, the clinically used RBE is assumed to have a fixed value of 1.1, mostly obtained from *in vitro* and *in vivo* measurements carried out under inconsistent and inadequately defined conditions. The use of a simple ratio is challenged. Cläre von Neubeck (German Cancer Consortium Partner Site Dresden and German Cancer Research Center, Heidelberg, Germany) pointed out that analyses of clinical response to assess RBE should take into account interpatient heterogeneity associated with the tumor (size, grade, location, infectious

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status, and mutations) and also preexisting medical conditions, life style habits, and other factors. Jeffrey Buchsbaum (NCI) pointed out that a major concern in proton therapy is the unanticipated severe toxicities that may be attributable to higher RBE at the distal and lateral beam edges. The suggested approach is a "continual reassessment model" to improve the understanding of the RBE variability based on data for populations of patients and with the help of artificial intelligence approaches to translate the knowledge continually into clinical applications. Roger Howell (Rutgers University, Newark, NJ) discussed that the RBE of targeted radionuclides that are widely used as standard of care to sterilize metastatic disease needs reevaluation. An interesting proposal is to formulate RBE in terms of 2 Gy equivalent fraction (EQD2) to relate it to conventional radiotherapy, but the type of radionuclide has great impact, for example, auger electron emitters can be more radiotoxic than alpha emitters (RBE as high as 8). Manjit Dosanjh (CERN, Geneva, Switzerland) emphasized the difference in protons and heavier ions with unique molecular and cellular responses compared with photons due to the complexity of DNA damage, differential gene expression, epigenetic modulation, and effect on cell cycle. Such differences will have profound clinical consequences.

Clearly, biomarkers and response predictors are needed to measure "radiation effects." The discussion in Session IV reviewed key issues including: optimal timing and source of the marker (tumor, biofluid, and imaging) and the need for reproducibility, standardization, and validation techniques including FDA approval. Biomarkers for organ injury may benefit from biodosimetry studies done for radiation incidents, although there are obvious differences in exposure. In both settings, predictive assays that accurately estimate the risk of injury to specific organ systems are of particular importance. Cytogenetic assays, including assessment of micronuclei and dicentric chromosomes, and γH2AX expression in lymphocytes are used. Newer biodosimetry approaches including metabolomics and quantifying circulating markers such as long noncoding RNA and miRNA may provide tumor response and organ-specific injury prediction (2–5). For therapeutic interventions, the goal is the integration of biomarkers of tumor and normal tissue response into a predicted biological outcome that allows personalization of the radiation treatment plan and dose appropriate for each patient based on the probability of individual organ toxicity and tumor control. Careful consideration should be given to circulating tumor cells (CTC) as a biomarker of tumor response, particularly in reference to local versus systemic recurrence, but also the use of other markers within the field of "liquid biopsy" such as ctDNA or exosomes.

Session V discussed the clinician's perspective on "dose" with outcomes and next generation of standard of care. Four speakers described topics of stereotactic body radiotherapy (SBRT)/hypofractionation (Bob Timmerman, University of Texas Southwestern Medical Center, Dallas, TX), RBE of proton therapy (Harald Paganetti, Massachusetts General Hospital), novel role of radiation for chemopotentiations (Iris Eke, NCI), and next generation of personalized radiation medicine (Quynh Le, Stanford University). Maximizing the therapeutic index was a key focus of these innovative strategies. With the delivery of high dose radiation in a hypofractionated manner for photons and particle therapy, the quality assurance of physical dose delivery precision in imaging, motion control, and dose modulation is of paramount importance because such high doses delivered in just a few fractions cannot depend on repair of normal tissues to maximize the therapeutic index. The potential role of SBRT in managing oligometastasis or oligoprogression exists but must be evaluated in randomized trials in order to understand its indications, limitations, and biological effects (6). New preclinical data were presented demonstrating that multifractionated radiotherapy was effective in upregulating integrin and mTOR/AKT signaling, leading to enhanced sensitivity to drugs induced by radiation, a proof of concept for the use of understanding the biological impact of radiation beyond dose (7). The use of patient-derived xenograft models to predict sensitivity to molecular-targeted therapies in combination with radiotherapy should be interrogated in a systematic manner in order to better understand their roles and limitations. Finally, there are trials evaluating personalized radiation using imaging, biology, circulating biomarkers such as ctDNA, and radiogenomics to maximize tumor control and/or to identify patients at risk for normal tissue toxicity (8, 9). To date, none can be applied in routine clinical practice.

With the general agreement that the biological description is critical in addition to physical dose, biological consequences of clinical relevance were discussed in Session VI. David Kirsch (Duke University, Durham, NC) summarized several technologies for the application of genetically engineered mouse models and described the importance of fully immune-competent models to the understanding of radiotherapy, immunotherapy, radiation-induced carcinogenesis, normal tissue injury, and the cell-autonomous and non–cell-autonomous mechanisms that contribute to normal and tumor tissue response to radiation. Phuoc Tran (Johns Hopkins University, Baltimore, MD) highlighted mechanisms by which radiation can enhance antitumor immune response, including increased expression of immune mediators and the development of micronuclei. Centrosome clustering can result in the selective killing of cancer cells with supernumerary centrosomes and increase in micronuclei by forcing cells into multipolar divisions. Drugs may enhance the cytotoxic effects of radiation while concurrently potentiating radiation-induced antitumor immunity through generation of micronuclei. Amit Maity (University of Pennsylvania, Philadelphia, PA) focused on combinations of molecular targeted agents with radiotherapy. Agents targeting the PI3K/Akt pathway affected EGFR and VEGF function to restore radiation sensitivity. Radiation combination clinical trials involving agents in DNA damage repair pathway inhibitors were noted, including inhibitors of ATM, ATR, DNA-PK, RAD51, WEE1, and PARP. Joanne Weidhaas (UCLA, Los Angeles, CA) discussed the genetics and biological consequences of miRNA germline mutations in radiation response. Genetic mutations in miRNA can alter radiation response. MiRNAs can inhibit oncogene signaling or enhance tumor suppressor targeting.

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MiRNA profiles can provide immunotherapy and radiotherapy biomarkers of toxicity and response.

Treating limited tumor volume and its impact on clinical outcome, including immunological consequences, were discussed in Session VII. There are preclinical data and clinical experience regarding the possibility to only treat a limited amount of the tumor or to use heterogeneous doses as a means of protecting normal tissue and eliciting intratumoral and systemic immune attack. The actual delivered radiation dose accounting for tumor motion and dose scatter must be fully understood. Chandan Guha (Albert Einstein College of Medicine, Bronx, NY) emphasized that small field radiotherapy may be sufficient to stimulate robust antigen presentation, allowing the host to develop an immune response and at the same time protect the T-cell recruitment process. Silvia Formenti (Cornell University, New York, NY) presented data that dsDNA is sensed by cGAS, which is needed to activate the IFN1 pathway via STING, but the complexity of the entire sensing-response system is critical, with 3 × 8 Gy being more effective in developing an immune response when combined with checkpoint immunotherapy, compared with a higher single dose of 30 Gy. The mechanism was mediated by Trex1 induced by the highest doses of radiotherapy that then reduces IFNγ1 activation. Robert Griffin (University of Arkansas, Little Rock, AR) reviewed some of the early findings using "grid" therapy (heterogeneous dose distribution) that was demonstrated in preclinical models. The observed alterations in gene expression and cell signaling were presumed to be related to a bystander effect. He pointed out that cell killing in this approach is reduced by hypoxia and that antiangiogenic agents might enhance cell killing. Xiadong Wu (University of Miami, Miami, FL) also discussed how partial tumor irradiation could alter the stromal components of the tumor and thus affect immunotherapy or other tumor effects. However, many of the stromal changes induced by partial or whole volume tumor irradiation are not well defined, and it is unclear how to best utilize these changes. In contrast to the work of Griffin, Wu suggested that it might be important to enhance, rather than suppress, angiogenesis.

The closing discussions in Session VIII focused on how to build on the experience and success from radiotherapy and biology using the current and rapidly emerging tools of precision medicine. New paradigms in radiation biology promise to transform the use of radiation in cancer therapy. One of the new paradigms (in some ways an old paradigm) is hypofractionation, in which biological equivalent dose escalation can improve outcomes for certain cancers. With the ability to target lesions precisely, hypofractionation is paving a new path for combined modality therapy. It has become almost routine using stereotactic techniques for disease sites such as lung, pancreas, and prostate and offers an intriguing opportunity with immunotherapy. Delivering less total dose than extended conventional radiotherapy using larger dose fractions, perhaps smaller volumes and short courses of treatment have sparked interest in immunotherapy.

There are many biological factors that might reasonably be expected to offer insights into how a course of radiation should be altered from traditional paradigms to improve local control rates. These include (a) pretreatment next-generation sequencing to estimate the probability of local control after radiotherapy, (b) real-time assessment of DNA repair using measurement of CTC γH2AX, (c) hypoxia-related resistance prediction during a course of therapy using serum HIF1α and CAIX, as well as imaging with oxygen-enhanced MRI or other contrast agents that can be used in PET, (d) real-time assessment of radiation response using periodic measurement of CTCs and ctDNA, and (e) pretherapy miRNA assessment, attempting to define patterns of radiation sensitivity and prognosis. Essentially, the goal of such a program is to "surround" the course of therapy with the assessment of multiple biological parameters that, over time, would provide the clinician with key information regarding appropriate dose, dose per fraction, fractionation scheme, and radiation target volume, thus allowing them to personalize therapy and to adapt to the dynamically changing tumor biology before and during therapy in order to obtain better outcomes.

The "Shades of Gy" paradigm offers unique opportunities and also requires rigorous assessment of the dose delivered as the dose in Gy as a physical "ground truth" to which biological changes must be related. Clinical radiotherapy is quite rigorous, taking into account organ motion, especially with hypofractionation. Further improvement is necessary as noted for the approaches employing heterogeneous doses and extremes of dose rate and size. The NCI Radiation Research Program has workshops planned in 2018 for targeted radionuclide therapy and GRID/Flash techniques. A new NCI UO1 grant program to study combinations of drugs plus radiation is now being implemented. The paradigm of radiation-inducible molecular targets has recently been demonstrated and needs further study. The understanding of the biology of particle therapy has been limited by the availability of beam time, but the new data on immune modulation and toxicity serve as an incentive for preclinical research. The interest in adding radiation to immunotherapy (such as checkpoint inhibitors) needs to be coupled with preclinical mechanism studies and biomarkers as being developed by immuno-oncologists. This "Shades of Gy" workshop is the initial presentation of this concept, with further dissemination forthcoming that proposes a "metastrategic plan" for radiation oncology and biology.

Disclosure of Potential Conflicts of Interest

D.G. Kirsch reports receiving commercial research grant from Eli Lilly, Merck, and XRAD Therapeutics, and has ownership interest (including patents) in XRAD Therapeutics. S.C. Formenti reports receiving commercial research grant from Bristol Myers Squibb, Eisai, Janssen, Merck, Regeneron, and Varian, has Honoraria from the Speakers' Bureau of Varian, and is a consultant/advisory board member for AstraZeneca, Bristol Myers Squib, Dynavax, Eisai, Elekta, GlaxoSmithKline, Janssen, Merck, and Regeneron. D. Raben is a consultant/advisory board member for Astra Zeneca, EMD Serono, Genentech, Merck, and Oncology Analytics. No potential conflicts of interest were disclosed by the other authors.

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